Journal of Surgical Oncology 1998;67:182–185

Adenoma With Clear Cell Change of the Large Intestine

HIROAKI SUZUKI, MD,[1,2]* SATOSHI OHTA, MD,[1] SHIROU TOKUCHI, MD,[3] JUN MORIYA,[2] YASUNORI FUJIOKA, MD,[2] AND KAZUO NAGASHIMA, MD[1]

[1]Second Department of Pathology, Hokkaido University School of Medicine, Sapporo, Japan

[2]Department of Pathology, Hokkaido University Hospital, Sapporo, Japan

[3]Aiiku Hospital, Sapporo, Japan

Background and Objectives: Clear cell change of the large intestinal neoplasm is rare, and its character remains unclear. We report a case of the large intestinal adenoma with clear cell change with immunohistochemical and molecular studies to investigate whether the clear cell change is associated with a malignant progression of the adenoma.

Methods: We studied the histochemical and immunohistochemical staining characteristics of the tumor by staining with hematoxylin-eosin, periodic acid-Schiff, alcian blue, and by immunostaining using antibodies against carcinoembryonic antigen, epithelial membrane antigen, p53, and Ki-67. The c-K-*ras* codon 12 point mutations were analyzed using a nonradioactive restriction fragment length polymorphism technique.

Results: The tumor was composed of a typical tubular adenoma and a tubular adenoma with clear cytoplasm. The clear cytoplasm was negative by mucin stains. Immunohistochemically p53 was negative in both the components. Labeling index of Ki-67 showed no significant difference between the two components. No codon 12 mutation of c-K-*ras* gene was observed in both the components.

Conclusion: These findings suggest that the clear cell change of the tubular adenoma is not associated with a malignant progression in adenoma-carcinoma sequence involving c-K-*ras* and p53.

J. Surg. Oncol. 1998;67:182–185.

KEY WORDS: adenoma; clear cell change; K-*ras*; p53; large intestine

INTRODUCTION

Clear cell epithelial neoplasms are rare in the large intestine [1–4]. Both benign and malignant forms have been reported, and the cells with clear cytoplasm were suggested to contain glycogen [1–4]. So far, several adenomas with clear cell change have been described [2–4]. We recently examined a case of tubular adenoma with clear cell change arising in the descending colon of a 62-year-old male. We report the pathologic findings with immunohistochemical and molecular studies to investigate whether the clear cell change is associated with a malignant progression of adenoma.

CASE REPORT

A 62-year-old Japanese male was found to have a pedunculated polyp in the descending colon during evaluation of occult bleeding. The polyp was removed endoscopically. Radiological examinations showed no evidence of tumor elsewhere in the body including the kidneys. The patient is alive, and the postoperative course was uneventful 12 months after the operation.

MATERIALS AND METHODS

Light Microscopy

The specimen was fixed in 10% formalin and embedded in paraffin. Sections (4 μm thick) were cut and

*Correspondence to: Dr. H. Suzuki, The Second Department of Pathology, Hokkaido University School of Medicine, N15W7, Kita-ku, Sapporo, 060, Japan.

Accepted 21 November 1997

stained using hematoxylin-eosin, periodic acid-Schiff (PAS), and alcian blue (pH 2.5).

Immunohistochemistry

Immunohistochemical studies were performed by the peroxidase avidin-biotin method using the formalin-fixed and paraffin-embedded material. The following primary antibodies were used: carcinoembryonic antigen (CEA, polyclonal, Kyowa Medex, Tokyo, Japan), epithelial membrane antigen (EMA, monoclonal, Dako, Glostrup, Denmark), p53 (monoclonal, Dako), and Ki-67 (monoclonal, Immunotech, Marseilles, France).

Polymerase Chain Reaction (PCR)-Restriction Fragment Length Polymorphism (RFLP) Analysis

Four 10 μm sections, in which the unnecessary portion was removed by scraping with a disposable scalpel blade, were processed for DNA extraction. DNA was extracted as previously described by Frank et al. [5]. Briefly, the dewaxed sections were incubated in 50 mM Tris, pH8.3, containing 200 ng/μl proteinase K at 37°C for 16 hr. Then, the solution in the microcentrifuge tube was immersed in boiling water for 8 min. The extracted DNA was placed on ice for 5 min and used for PCR. Lu65 cells (Japanese Cancer Research Resource Bank) were used as positive control [6,7]. Normal splenic tissue was used as a negative control. PCR-RFLP analysis was based on the method of Jiang et al. [8]. The primers were prepared as follows [8,9]:

K-ras 5′: 5′ ACTGAATATAAACTTGTGGTAGTTG-GACCT 3′
K-ras 3′: 5′ TCAAAGAATGGTCCTGGACC 3′

PCR was performed using thermal cycler 2400 (Perkin-Elmer Cetus, Emeryville, CA), in a final volume of 25 μl containing template DNA (2 μl), 50 mM KCl, 10 mM Tris-HCl (pH8.3), 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.5 μM of each primer, and 0.625 U of Taq gold polymerase (Perkin-Elmer Cetus). The PCR amplification conditions were 10 min at 95°C, followed by 35 cycles with 1 min at 95°C, 2 min at 50°C, 1 min at 72°C. The primers used in this analysis produce a fragment that is 157 base pairs (bp) long [8,9]. Digestion of the PCR products with Bst NI (Mva I, Toyobo, Tokyo, Japan) results in a PCR fragment of 143 bp if a mutation is present in codon 12 of K-*ras* gene and 114 bp fragment if no mutation is present [8,9]. The samples were electrophoresed through 12% polyacrylamide gel. Gels were stained with ethidium bromide and photographed on an ultraviolet light transilluminator.

RESULTS

Pathological Findings

Gross and microscopic findings. The knobby tumor removed from the descending colon measured 1.4 × 1.2 × 1.0 cm. Microscopically, the polyp consisted of two intermixed components (Figs. 1 and 2). Approximately 40% of the tumor was a dark component showing typical adenomatous tubular glands with mild to moderate atypia. Approximately 60% of the tumor was a clear component showing tubular glands that consisted of clear cells with moderate to severe atypia. The clear cells had abundant clear vacuolated cytoplasms (Fig. 3), and they were negative with periodic acid-Schiff (PAS) both before and after diastase digestion. The clear cells were also negative with alcian blue staining. However, the typical adenomatous tubular glands were positive with PAS both before and after diastase digestion and positive with alcian blue staining.

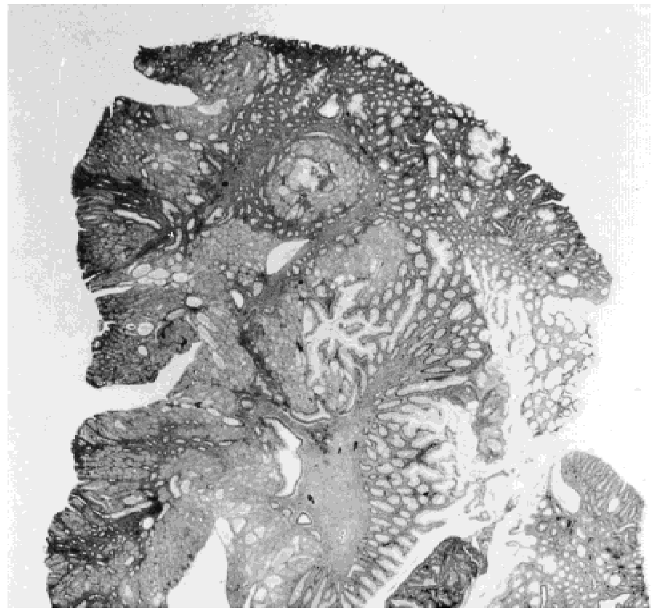

Fig. 1. Low magnification of the tumor. Clear cell component and dark cell component were intermixed (H&E stain, ×7.5).

Immunohistochemistry

Both the clear and dark components were strongly positive for CEA in the cytoplasm and cell membrane. EMA was weakly immunopositive in both the components. Overexpression of p53 protein was not observed in either component. Ki-67 labeling index (LI, %) was calculated by counting the positive nuclei per 2,000–3,000 cells of both components. There was no significant difference between the two components, although the clear cell component showed slightly higher Ki-67 LI than the dark component, i.e., LI 71.6% vs. 63.8%.

PCR-RFLP Analysis of the K-*ras* Gene

As shown in Figure 4, undigested PCR products of both components showed the expected band of 157 bp and PCR products of both the components digested with BstNI demonstrated only 114 bp fragments. Positive con-

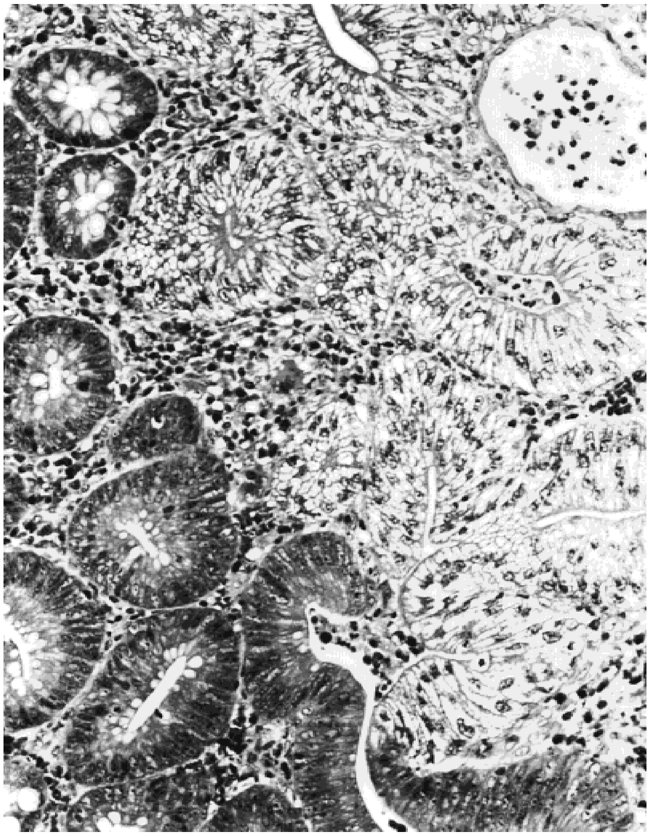

Fig. 2. Photomicrograph of the tumor. One tubular gland consists of both the components (H&E stain, ×100).

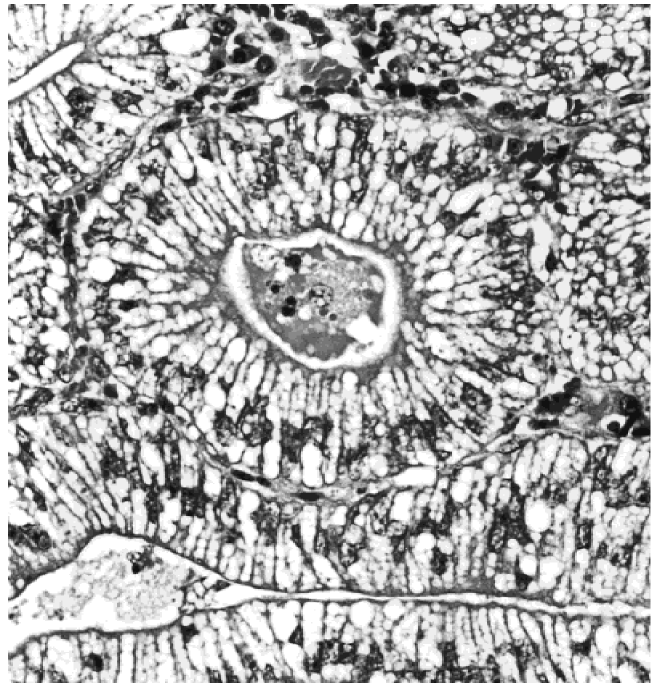

Fig. 3. Photomicrograph of the clear cell component of the tumor (H&E stain, ×400).

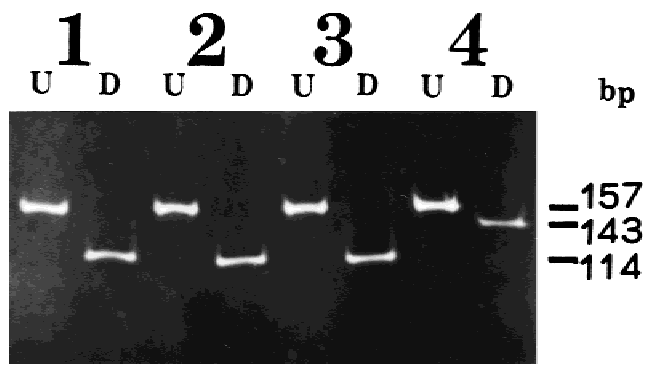

Fig. 4. PCR-RFLP analysis of c-K-*ras* codon 12 in the tumor. U, undigested; D, digested with BstNI; 1, clear cell component of the tumor; 2, dark component of the tumor; 3, spleen; 4, Lu-65 cell line; bp, base pair.

trol (Lu-65) showed 143 bp fragment after digestion with BstNI. These results indicated the absence of any c-K-*ras* codon 12 mutations in both components.

DISCUSSION

Clear cell epithelial neoplasms of the large intestine appears to be extremely rare [1–4], and so far only two cases have been reported as benign clear cell neoplasms [2–4]. Both cases were adenomatous polyps showing clear cell component partially, and it was thought to be a clear cell change of the tubular adenoma. Our case appeared to be the same type of tumor as these two cases.

The major diagnostic problem may be confusion with metastatic renal cell carcinoma [2–4]. Reed et al. [2,4] described that the immunoperoxidase reaction for CEA is helpful for this problem because CEA is immunonegative in the renal cell carcinoma. Our case also showed that both components were immunopositive for CEA, suggesting that the clear component is also a primary lesion. Previous reports suggested that the clear nature of the cytoplasm was due to glycogen [1–4]. In our case, the clear component was also negative with PAS, as well as with alcian blue staining, indicating that at least clear cells contain no mucin.

As described in Results, the clear cell component is morphologically very different from the dark cell component. Thus it is interesting to study whether the clear cell component shows a genetic malignant progression compared with the dark cell component. Recently, the adenoma-carcinoma sequence of colon cancer has been studied, and genetic alterations of the c-K-*ras* oncogene and p53 suppressor gene are thought to be implicated in this sequence [10]. The codon 12 mutation of c-K-*ras* oncogene has been observed more frequently in large adenomas than in small adenomas, suggesting that the codon 12 mutation is associated with a malignant progression of the adenoma [10]. Both components of our case showed no codon 12 mutation of c-K-*ras* gene. Ab-

normal overexpression of the p53, which is frequently caused by its somatic mutation, was not observed in both components. As Ki-67 LI has been found to correlate with cell proliferation and degree of tumor malignancy [11,12], Ki-67 LI was counted on both the components. Although the clear cell component showed slightly higher LI of Ki-67 compared with the dark cell component, the difference was small. Taken together, the clear cell change itself appeared to have no association with a malignant progression in adenoma-carcinoma sequence involving the c-K-*ras* and p53. However, more molecular biological studies may be necessary to elucidate this association, because other genetic alterations including APC and DCC genes were observed in the adenoma-carcinoma sequence in addition to the changes of K-*ras* and p53 genes [10].

REFERENCES

1. Hellstrom HR, Fisher ER: Physaliferous variant of carcinoma of colon. Cancer 1964;17:259–264.
2. Reed RJ, Love GL, Harkin JC, et al.: Consultation case. Am J Surg Pathol 1983;7:597–601.
3. Jewell LD, Barr JR, McCaughey WTE, et al.: Clear-cell epithelial neoplasms of the large intestine. Arch Pathol Lab Med 1988;112: 197–199.
4. Lewin KJ, Riddell RH, Weinstein WM (eds): ''Gastrointestinal Pathology and Its Clinical Implications.'' New York: Igaku-Shoin, 1989.
5. Frank TS, Svoboda-Newman SM, His ED: Comparison of methods for extracting DNA from formalin-fixed paraffin sections for nonisotopic PCR. Diagn Mol Pathol 1996;5:220–224.
6. Taya Y, Hosogai K, Hirohashi S, et al.: A novel combination of K-ras and myc amplification accompanied by point mutational activation of K-ras in a human lung cancer. EMBO J 1984;3: 2943–2946.
7. Suzuki Y, Orita M, Shiraishi M, et al.: Detection of ras gene mutations in human lung cancers by single-strand conformation polymorphism analysis of polymerase chain reaction products. Oncogene 1990;5:1037–1043.
8. Jiang W, Kahn SM, Guillem JG, et al.: Rapid detection of ras oncogenes in human tumors: Application to colon, esophageal, and gastric cancer. Oncogene 1989;4:923–928.
9. Mizuuchi H, Mori Y, Sato K, et al.: High incidence of point mutation in K-ras codon 12 in carcinoma of the fallopian tube. Cancer 1995;76:86–90.
10. Crawford JM: The gastrointestinal tract. In Cotran RS, Kumar V, Robbins SL (eds): ''Pathologic Basis of Disease.'' Philadelphia: Saunders, 1994, p 814–815.
11. Beck T, Weller EE, Weikel W, et al.: Usefulness of immunohistochemical staining for p53 in the prognosis of breast carcinomas: Correlations with established prognosis parameters and with the proliferation marker, MIB-1. Gynecol Oncol 1995;57:96–104.
12. Barbareschi M, Girlando S, Mauri FM, et al.: Quantitative growth fraction evaluation with MIB-1 and Ki67 antibodies in breast carcinomas. Am J Clin Pathol 1994;102:171–175.